

AZERBAIJAN MEDICAL UNIVERSITY DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

LESSON 15.

Microbiology diagnosis of acute respiratory viral infections (families of Adenoviridae and Coronaviridae, Rhinovirus genus) and smallpox (Poxviridae family)

FACULTY: General Medicine SUBJECT: Medical microbiology - 2

Discussed questions:

1. Adenoviridae family and general properties of human adenoviruses (morpho-biological properties, antigenic properties, classification, diseases caused by them. Microbiological diagnosis of adenovirus infections.

2. Family Coronaviridae, general properties, diseases they cause and microbiological diagnostics.

3. Rhinovirus genus, general properties, diseases caused by them and microbiological diagnostics.

4. Poxviridae family, general properties. Monkeypox virus. Pathogenicity characteristics. Microbiological diagnosis.

Purpose of the lesson:

 To acquaint students with the morpho-biological characteristics of adenoviruses, coronaviruses, rhinoviruses and poxviruses and to provide information on laboratory diagnostic methods of diseases caused by these viruses.

ADENOVIRUSES





- Adenoviridae is a group of medium sized, nonenveloped, double stranded DNA viruses that replicate and produce disease in the eye and in the respiratory, gastrointestinal and urinary tracts;
- Originally isolated from adenoids;
- At least 47 serotypes associated with human diseases;
- Most infections asymptomatic;
- Infections are common in children;

MORPHOLOGY

- Icosahedral, about 80-110 nm diameter;
- 252 capsomeres;
- Fibre projects from each of 12 vertices;
- · Resemble a space ship;
- Genome is dsDNA;
- Replicates in nucleus;





CLASSIFICATION

- Family Adenoviridae : 2 genera
 - Aviadenovirus : adenoviruses of birds;
 - Mastadenovirus : infect mammals;
 - 47 serotypes of human origin;
 - 6 subgroups (A-F) based on genome homology;
 - Subgroup A : serotypes 12,18 and 31 : highly oncogenic;

DISEASE ASSOCIATIONS

Serotype	Disease	At risk
1-7	Acute febrile pharyngitis	Children
3,7,14	Pharyngoconjunctival fever	Older children
8,9,37	Epidemic keratoconjunctivitis (shipyard eye)	Adults
3,4,11	Acute follicular conjunctivitis	Any age
40,41	Diarrhoea	Infants, young children
11,21	Hemorrhagic cystitis	Children

VIRAL REPLICATION

- Adenoviruses attach to surface of the cells by their fibers, then penetrate the cell, and once inside the cell, uncoat the viral DNA.
- The viral DNA is then transported into the nucleus of the cell and initiates replication cycle.
- Host cell DNA-dependent RNA polymerase transcribes the early genes leading to formation of functional mRNA.



VIRAL REPLICATION



- Then in the cytoplasm, the early mRNA is translated into nonstructural proteins.
- In the nucleus, after viral DNA replication, late mRNA is transcribed and then translated into structural virion proteins.
- This is followed by assembly of virions in the nucleus and release of virions by lysis of the cells, but not by budding.

- Adenoviruses are transmitted mainly by respiratory or feco-oral contact from humans.
- They infect the conjunctiva or the nasal mucosa.
- They may multiply in conjunctiva, pharynx, or small intestine, and then spread to preauricular, cervical and mesenteric lymph nodes, where epithelial cells are infected.



respiratory transmission





6 cal-oral transmission

- Adenoviruses may cause three different types of interaction with the infected cells.
- These are (a) lytic infection, (b) latent infection, and (c) transforming infection.
- lytic infection: Adenoviruses infect mucoepithelial cells in the respiratory tract, gastrointestinal tract, and conjunctiva or cornea, causing damage of these cells directly.
- After local replication of the virus, viremia follows with subsequent spread to visceral organs.
- Dissemination occurs more commonly in immunocompromised patients than in the immunocompetent individuals.

- latent infection: The adenovirus has a unique ability to become latent in lymphoid and other tissues such as adenoids, tonsils, and Payer's patches.
- The exact mechanism of latency of adenoviruses in these tissues is not known.
- These latent infections can be reactivated in patients infected with other agents or in the patients who are immunocompromised.

- Oncogenic transformation: Some adenoviruses belonging to groups A and B have the property for oncogenic transformation in rodent cells.
- During oncogenesis the multiplication of adenovirus is inhibited followed by integration of viral DNA into the host DNA.
- After integration adenoviruses produce E1A proteins which target rodent cells by altering cellular transcription, finally leading to transformation of rodent cells.
- However, oncogenesis of human cells has not been demonstrated.

- Adenoviruses primary infect children.
- Adults are also infected.
- More than one serotypes of virus may produce the same clinical syndrome and one serotype of virus may cause clinically different diseases.
- Adenoviruses 1-7 are the common serotypes worldwide and are responsible for most cases of adenovirus-associated infections.
- Incubation period varies from 5 to 8 days.
- The major clinical syndromes caused by the human adenoviruses are as follows.

- Respiratory diseases
- Acute respiratory diseases: This disease is caused by adenovirus serotypes 4 and 7.
- Fever, rhinorrhea, cough, and sore throat are the typical symptoms which last for 3-5 days.
- This syndrome most often affects military recruits living in crowded conditions.
- Pharyngoconjunctival fever: This syndrome occurs primarily in school-going children.
- Fever, sore throat, coryza, and red eye are the classic presentations of the condition.



- These symptoms may precede ocular findings, or they may not be present.
- Acute conjunctivitis may occur as a separate entity with or without pharyngitis.
- The condition is usually self-limiting.
- This condition is caused predominantly by serotypes 3, 4, and 7.
- Other respiratory tract diseases: Bronchiolitis, croup, laryngitis, and cold-like symptoms are the other respiratory tract diseases caused by adenoviruses.

- Epidemic keratoconjunctivitis:
- This is a highly contagious condition and has an insidious onset of unilateral red eye.
- Subsequently, both the eyes are involved.
- Patients complain of photophobia, tearing, and pain.
- Fever and lymphadenopathy may be present in some children.

- Inflammation of the conjunctiva may persist for a week, accompanied with residual scarring and visual impairment.
- The condition may occur as an epidemic, which is usually caused by serotype 8 and less often by serotypes 19 and 37.



- Gastroenteritis and diarrhea
- These conditions are most commonly associated with serotypes 40 and 41, but other serotypes may also be involved.
- The entecic adenovirus infection is a common cause of infantile diarrhea in day-care centers.
- The condition manifests as fever and watery diarrhea, which resolves within 1-2 weeks.
- Other syndromes :
 - Acute hemorrhagic cystitis (11,21)
 - Generalised exanthems
 - Mesenteric adenitis
 - Intussusception

LABORATORY DIAGNOSIS

- Specimens : from throat, eye, urine, feces;
- Isolation of virus:
 - Inoculation into cell cultures;
 - human embryonic kidney/HeLa/HEp2
 - CPE : cell rounding and aggregation into grape like clusters;
 - Other tests : HA, Neutralization, CF

LABORATORY DIAGNOSIS

- Serology : rise in titre of antibodies in pared sera; (single specimen – not useful)
- Electron Microscopy : for stool to see virus
- Immunofluorescence : antigen detection in Nasopharyngeal /occular specimens



- Specific prevention only for control of outbreaks in closed communities;
- Killed and live vaccines tried in Acute Respiratory Disease(ARD);
- No vaccine for general use;



Classification :

- * Family: Coronaviridae
- *•• Gender: Coronavirus
- *• Genome: linear single-stranded RNA +
- * pleomorphic, Wrapped
- 💠 80 to 220 nm
- 30 serotypes





Reproductive scheme of coronaviruses

What Do We Know About Coronaviruses?

Coronaviruses have a crown-like appearance under the microscope

Coronaviruses are a large family of viruses some cause illness in people, and others only infect animals.

Some coronaviruses infect animals then spread to people, and then spread person to person such as:

- Middle East Respiratory Syndrome (MERS)
- Severe Acute Respiratory Syndrome (SARS)
- Coronavirus Disease 2019 (COVID-19)

Common coronaviruses include some that cause mild upper-respiratory illnesses, like the common cold.

What is COVID-19?

Coronavirus disease 2019 or COVID-19 is a respiratory illness that can spread from person to person.

The virus that causes COVID-19 is a **new** coronavirus first identified during an investigation into an outbreak in Wuhan, Hubei Province, China.

Initial case-patients reported visiting a large seafood and live animal market in Wuhan.

CROWNE PLAZA

Symptoms

- Corona viruses primarily infect the upper respiratory and gastrointestinal tract of humans.
- Most people who got infected with the novel corona virus developed severe acute respiratory illness with symptoms of fever, cough, and shortness of breath. Some people were reported as having a mild respiratory illness

Microbiological diagnosis of coranovirus infections

- Viruses can be detected in respiratory secretions by ELISA, IFR and PCR.
- RNA for SARS and Covid-19 virus can also be found in the blood.
- Since the virus is difficult to obtain in cell cultures, the main diagnostic method is PCR.
- An increase in the antibody titer in the double serum studied by ELISA confirms coronavirus infection.

Testing, testing

Promising drugs to treat covid-19

Drug	Current use	Original mode of action	Being tested?
Chloroquine	Antimalarial	Heme polymerase inhibitor	Yes
Kaletra (ritonavir + lopinavir)	HIV	Protease inhibitor	Yes
Interferon alfa-2b	Hepatitis-C	Immune modulator	Yes
Remdesivir	Experimental	Nucleotide analogue	Yes
Favipiravir	Influenza	RNA polymerase inhibitor	Yes
Actemra (tocilizumab)	Rheumatoid arthritis; covid-19	Anti-inflammatory	Approved*
Kevzara (sarilumab)	Rheumatoid arthritis	Anti-inflammatory	Trials expected

Source: WHO, adapted from landscape analysis, 17th February 2020

*For use on covid-19 in China, March 2020

Rhinoviruses

 Rhinoviruses (from the Greek (gen.) "nose") are the most common viral infective agents in humans and are the predominant cause of the common cold. Rhinovirus infection proliferates in temperatures between 33–35 °C (91–95 °F), and this may be why it occurs primarily in the nose. Rhinovirus is a species in the genus Enterovirus of the **Picornaviridae** family of viruses.

Rhinovirus

- Picornoviridae family
- Size
- Single stranded
- Incubation period of 1 to 3 days
- Optimum growth occurs between 33 and 34 deg Celsius (93 deg F)
- Not stable below the pH of 5-6

Rhinoviruses are ..

- Rhinoviruses are the most commonly isolated viruses from persons with mild upper respiratory illness.
- Rhinoviruses are a genus of picornaviridae
- In contrast to enteroviruses they do not replicate in the intestinal tract, they have an extreme species specificity and more fastidious growth requirements

Properties of Rhinoviruses.

- Gross appearance like Entero viruses,
- Acid Labile destroyed at 3.0 pH.
- Grown in

Human Cell lines, WI – 38 MRC -5

Cultivated at 33⁰ c

Transmission of Rhinoviruses

 There are two modes of transmission: via aerosols of respiratory droplets and from contaminated surfaces, including direct person-toperson contact.

Pathogenesis **Epithelial destruction hypothesis**:

intact nasal epithelium (Winther et al)

rhinovirus replication in extremely small number of cells (Arrunda et al)

viral quantity does not influence duration nor severity of colds (Arrunda et al) Dr.TV.Rap MD

Pathogenesis - Rhinoviruses

- Entry through Respiratory tract.
- Nasal Mucosa, can infect Lower Respiratory tract.
- Chilling, wearing wet cloths do not produce infection.
- But common cold starts with chills.
- Local inflammation and cytokines may be responsible for the symptoms of common cold.
- Interferon production occurs early and specific antibody appears in nasal secretions

Laboratory Diagnosis

- Isolation of virus may be obtained from nasal or throat swabs collected early in infection.
- Culturing on MRC5 or W 138
- Appearing of CPE DETURE

Prevention and Control

- No specific treatment.
- Vaccines are unsuccessful.
- Intranasal spray of Gamma Interferon for 5 Days

Poxviridae family

- The family Poxviridae (lat., pox pustula) consists of large-sized, complexstructured DNA-containing viruses that cause diseases in insects and vertebrates.
- Viruses that cause diseases in vertebrates belong to the subfamily Chordopoxvirinae.
- Four of the six genera of this subfamily Orthopoxvirus, Parapoxvirus, Molluscipoxvirus and Yatapoxvirus cause various diseases in humans.
- Orthopoxvirus genus includes vaccinia virus, natural smallpox virus, viruses of cows, monkeys, camels, mice, etc. including smallpox viruses.
- Parapoxviruses include Orf (ecthyma contagion), bovine papulosis stomatitis virus, paravaccin (false cowpox) virus, etc. includes.
- Molluscipoxvirus genus includes molluscum contagiosum virus.Yaba virus and Tana virus from the genus Yatapoxvirus are pathogenic viruses for humans.

Poxviridae family

• Poxviruses are the largest viruses, measuring 230x400 nm. The virion is ovoid in shape, composed of a membrane, an outer membrane and a core (DNA and proteins) located between the lateral bodies. the virion's genome consists of double-stranded linear DNA. Reproduction takes place only in the cytoplasm, Guarinieri appendages are observed inside the infected cells. Virions bud from the plasma membrane and are released when the cell lyses.

Is MonkeyPox the next pandemic ?

Monkeypox

- Monkeypox virus is an enveloped double-stranded DNA virus with a genome size of around 190 kb.
- Monkeypox is a viral zoonotic disease that occurs primarily in tropical rainforest areas of Central and West Africa and is occasionally exported to other regions.

Structure

MONKEYPOX

HOW IT SPREADS

Primarily animals, typically rodents

Scratches

Bites

but limited

secretions or skin lesions

Confirmed human monkeypox cases Africa, 1970-2021

Confirmed human monkeypox cases Worldwide, 1970-2021

Monkeypox transmission - an overview

Monkeypox – modes of transmission

- Unprotected contact with:
- respiratory droplets
- lesion material
- body fluids
- contaminated materials and surfaces The virus can enter through:
- respiratory tract
- mucous membranes (eyes and mouth)
- broken skin (e.g. animal bites

Disease progression I – Incubation

- Duration: 5-21 days
- No symptoms
- Virus present in bloodstream (viremia) at the end of the incubation period

Disease progression II – Febrile stage

- 1-4 days
- Fever + other symptoms: swollen lymph nodes (lymphadenopathy), headache, chills, sore throat, malaise, fatigue
- Virus in the blood
- Small lesions in the mouth (enanthem) appear towards the end

Disease progression III - Rash stage

Credit: Emerg Infect DIS / N. Erez et al., 2018. Retrived from:<u>https://wwwnc.cdc.gov/</u> eid/article/25/5/19-0076-f1

Disease progression III – Rash stage

- Virus may be in the blood early in this stage
- Virus is present in skin lesions
- Antibodies are produced and become detectable

Disease progression IV – Recovery

- The patient has recovered
- Specific antibodies are present in the blood
- Scars may remain

Monkeypox and other common rash illnesses

		Monkeypox	Chickenpox	Measles
Symptoms	Fever	1-3 days before rash	1-2 days before rash	3-5 days before rash
	Rash appearance	Lesions often in one stage of development	Lesions often in multiple stages of development	Lesions often in multiple stages of development
	Rash development	Slow	Rapid	Rapid
	Rash distribution	More dense on face; present on palms and soles	More dense on trunk; Absent on palms and sole	Starts on face and spreads, sometimes reaching hands and feet
	Lymphadenopathy	Present	Absent	Occasional
	Death	Up to 10%	Rare	Varies widely

Clinical Symptoms

- Monkeypox typically presents clinically with fever, rash and swollen lymph nodes and may lead to a range of medical complications.
- Monkeypox is usually a self-limited disease with the symptoms lasting from 2 to 4 weeks.
- Severe cases can occur.
- In recent times, the case fatality ratio has been around 3-6%.
- Monkeypox is transmitted to humans through close contact with an infected person or animal, or with material contaminated with the virus.
- Monkeypox virus is transmitted from one person to another by close contact with lesions, body fluids, respiratory droplets and contaminated materials such as bedding.

Prevention

- Raising awareness of risk factors and educating people about the measures they can take to reduce exposure to the virus is the main prevention strategy for monkeypox.
- Scientific studies are now underway to assess the feasibility and appropriateness of vaccination for the prevention and control of monkeypox.
- Some countries have, or are developing, policies to offer vaccine to persons who may be at risk such as laboratory personnel, rapid response teams and health workers.

Treatment

• At this time, there are no specific treatments available for be monkeypox infection, but monkeypox outbreaks can controlled.

- Smallpox vaccine, cidofovir, ST-246, and vaccinia immune globulin (VIG) can be used to control a monkeypox outbreak.
- CDC guidance was developed using the best available information about the benefits and risks of smallpox vaccination and drug use for the prevention and management of monkeypox and other orthopoxvirus infections.

Monkeypox and Smallpox Vaccine

- One vaccine, JYNNEOS[™] (also known as Imvamune or Imvanex), has been licensed in the United States to prevent monkeypox and smallpox.
- Because monkeypox virus is closely related to the virus that causes smallpox, smallpox vaccine can also protect people from getting monkeypox.
- Past data from Africa suggests that smallpox vaccine is at least 85% effective in preventing monkeypox.
- The effectiveness of JYNNEOS[™] against monkeypox was concluded from a clinical study on the immunogenicity of JYNNEOS and efficacy data from animal studies.
- Experts also believe that vaccination after a monkeypox exposure may help prevent the disease or make it less severe.